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Distinct Regions in Human T-Cell Lymphotropic Virus Type I Tax Mediate Interactions with Activator Protein CREB and Basal Transcription Factors

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Human T-cell lymphotropic virus type I (HTLV-I) transactivator Tax augments transcription from three (cyclic AMP response element (CRE)-containing 21-bp repeats in the viral long terminal repeat and several other *cis* regulatory elements, including the NF-κB binding sites and the serum response element. Tax does not bind DNA directly; rather, it acts via cellular sequence-specific DNA binding proteins to stimulate transcription. We have shown recently that Tax forms multiprotein complexes with the heterodimeric and homodimeric forms of a ubiquitous cellular transcription factor, CREB (CRE binding protein). In vitro selection for preferred Tax-CREB binding sites indicates that the Tax-CREB complex exhibits greatly increased DNA recognition specificity and assembles preferentially on CRE motifs, TGACGT/C, flanked by long runs of G (5′) and/or C (3′) residues, as found in the HTLV-I 21-bp repeats. The indirect tethering of Tax to the 21-bp repeats via CREB is crucial for Tax transactivation. We now report the domain organization of Tax by characterizing its mutants. Tax mutants with alterations in the NH_2 terminus, including three deletion mutants, Tax(6–353), Tax(21–353), and Tax(89–353), and two amino acid substitution mutants, M1 (H3S) and M7 (C29A, P30S), all failed to interact with CREB in vitro. In contrast, a short COOH-terminal deletion, Tax(1–319), and a Tax mutant with amino acid substitutions near the COOH end, M47 (L319R, L320S), were able to interact with CREB and the 21-bp repeats to assemble ternary Tax-CREB-DNA complexes. As demonstrated earlier, M1, M7, and M47 all failed to transactivate the HTLV-I long terminal repeat. Our data indicate that the defects in M1 and M7 result from an inability to interact with CREB. In contrast, the COOH-terminal mutations in M47 most likely inactivated the transactivation domain of Tax. As anticipated, a Tax mutant, M22 (G137A, L138S) which activated transcription from the 21-bp repeats with reduced capacity and was defective in *trans* activating the NF-κB binding sites, continued to interact with CREB in vitro, albeit with a lower level of efficiency. Finally, a glutathione *S*-transferase (GST)-Tax fusion protein with the GST moiety fused to the NH_2 terminus of Tax failed to interact with CREB. Removal of the GST domain from GST-Tax by thrombin restores Tax's ability to assemble a ternary Tax–CREB–21-bp-repeat complex. These data support the notion that the NH_2-terminal region of Tax is important for interaction with CREB while the COOH end of Tax most likely is involved in communication with the basal transcriptional machinery.

Human T-cell lymphotropic virus type I (HTLV-I) is the causative agent of adult T-cell leukemia/lymphoma (20, 32) and a neurologic disorder called tropical spastic paraparesis/HTLV-I-associated myelopathy (3, 15, 24, 30). The 3′ region of the HTLV-I genome encodes a 40-kDa nuclear protein, Tax, crucial for virus replication (7, 23). Tax is a transcriptional activator. It activates HTLV-I transcription from three cyclic AMP response element (CRE)-containing 21-bp repeats in the U3 region of the viral long terminal repeat (LTR) (4, 9, 13, 27, 34, 39). In addition, it induces transcription of human immunodeficiency virus, interleukin 2, interleukin 3, granulocyte macrophage colony-stimulating factor (GMCSF) and interleukin 2 receptor α-chain genes via the NF-κB binding motif (8, 22, 26) and expression of the immediate-early gene, c-*fos*, via the serum response element (11). Tax does not bind DNA directly (17, 29). Recent evidence indicates that Tax function is mediated through direct interactions with cellular transcription factors. We demonstrated earlier that Tax interacts with the ubiquitous transcription factor CRE binding protein (CREB) to form a multiprotein complex which assembles specifically on the 21-bp repeats in the U3 region of the HTLV-I LTR (42, 43). Association of Tax with two members of the NF-κB/Rel family of transcriptional factors, p105 (p50 precursor) (21) and p100 (lyt-10, p52 precursor) (2, 25), has also been reported. The specific mechanism responsible for Tax-mediated activation from the NF-κB binding motifs is not fully resolved. It appears that, in the presence of Tax, the nuclear localization of various NF-κB factors, including c-*rel*, p65, p50, and p52, becomes greatly facilitated (8, 25). Most recently, Tax has also been shown to associate with $p67^{SRF}$, a cellular transcription factor mediating serum activation of c-*fos* expression (10, 11). This interaction of Tax with SRF correlates with activation of c-*fos* promoter by Tax (11).

Much like other eukaryotic virus transcription activators, such as herpes simplex virus VP16, Tax appears to contain multiple domains involved in interacting with various cellular effector proteins. By linker-scanning mutagenesis, Smith and Greene (36) previously demonstrated that distinct regions in Tax were involved in activating transcription from the CRE containing the HTLV-I LTR and the NF-κB binding site containing the human immunodeficiency virus type 1 LTR. From a total of 51 Tax mutants, 3 were found to activate exclusively via either the CREB-ATF pathway or the NF-κB pathway. Mutants M1 (H3S) and M47 (L319R, L320S) were defective in

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transactivating the HTLV-I LTR but continued to activate the human immunodeficiency virus type 1 LTR, while mutant M22 (G137A, L138S) activated the HTLV-I LTR but not the human immunodeficiency virus type 1 LTR. Several mutants with NH_2-terminal alterations, including M7 (C29A, P30S), were impaired in nuclear localization. The Tax domain necessary for nuclear transport has been localized to amino acid residues 1 to 48 (18, 38). In addition, the NH_2 terminus of Tax has been implicated in determining the specificity for the transcriptional activation of the HTLV-I or HTLV-II viral LTRs by the respective transactivators (5). Whereas these studies hinted at the importance of the NH_2-terminal domain of Tax in nuclear localization and promoter selectivity, fusion of different regions of Tax to the Gal4 DNA binding domain suggests that a transactivation domain exists in the COOH terminus of Tax between residues 284 and 325 (12, 35). In this study, we describe the in vitro characterization of Tax mutants. Our results indicate that the integrity of the NH_2 terminus of Tax is critical for interaction with CREB. This interaction was aborted by deletions, amino acid substitutions, or steric hindrance imposed by the glutathione *S*-transferase (GST) domain. In contrast, the COOH-terminal region of Tax, albeit critical for Tax transactivation, could be removed without severely affecting Tax–CREB–HTLV-I 21-bp-repeat ternary complex assembly. This region most likely serves to interact with the basal transcriptional machinery to affect initiation of mRNA synthesis. These results support the presence of a multidomain structure of the Tax protein and define biochemically the functional defects associated with the previously identified Tax mutants.

MATERIALS AND METHODS

Plasmid constructs. The cDNA for CREB was cloned at the *Nde*I-*Bam*HI sites of the pET-11a expression plasmid under the control of the bacteriophage T7 promoter as reported earlier (43). CREB cDNA contained in a 1-kb fragment with a blunted *Nde*I site at the 5′ end and a *Bam*HI site at the 3′ end was inserted into plasmid pGEX2T at *Sma*I and *Bam*HI sites to yield the expression plasmid pGST-CREB for the GST-CREB fusion protein. The recombinant plasmid was transformed into HB101 cells, and the fusion protein GST-CREB was synthesized after IPTG (isopropyl-β-D-thiogalactopyranoside) induction. The GST-CREB fusion protein was then purified from glutathione-agarose beads after elution with glutathione. The presence of CREB in the fusion protein was confirmed by immunoblot analysis and gel shift assay (not shown). The GST-Tax fusion construct was generated as GST-CREB, except that the TaxH6 coding sequence was contained in a DNA fragment with a blunted 5′ *Nco*I site and staggered 3′ *Bam*HI site. GST-Tax(96–353) was constructed by ligating the PCR-generated Tax sequence with the forward primer (5′) CGCGGATCCCA TACAACCCCC and the reverse primer (5′) TTGAGCCATATGTTAATTA ATGGTGGTGATGGTGGTGGACTTCTGTTTCT CGGAA (oligo-TaxH6) into the pGEX2T vector. GST-M1 was constructed by substitution of the *Nco*I-*Cla*I fragment in the GST-Tax with the corresponding region from pET-M1 and subsequently confirmed by sequencing. GST-M7 was constructed by substitution of the *Acc*I-*Cla*I fragment in the GST-Tax with the corresponding region from the cytomegalovirus (CMV) vector CMV-M7. Expression of all Tax fusion proteins was confirmed by Western blotting (immunoblotting).

The various NH_2-terminal deletions of Tax were constructed by incorporation at specific positions in the Tax coding sequence of ATG initiation codons contained within an *Nco*I site by PCR, followed by cloning of the truncated Tax sequences into pET-11d plasmid via *Nco*I-*Bam*HI sites. Plasmid pX5 harboring the Tax cDNA under the control of the bacteriophage T5 P21 promoter (16) was linearized with *Eco*RI and used as the template. The sequences of the oligonucleotide primers used in the PCR are as follows. The reverse primer oligo-TaxH6 was used in all PCRs. Forward primers for Tax(6–353), Tax(21–353), and Tax(89–353) are (5′) CGCGGATCCATGGGTTTGGACAGAGTC, (5′) GCA TGCCATGGGAGACTGTGTACAAG, and (5′) GCATGCCATGGTCCTTA CCCCGCCA, respectively. The DNA fragments containing amino acid substitutions in mutant Tax, M7, M22, and M47, were derived from PCR amplification with mutant Tax cDNA sequences present in a CMV-based eukaryotic expression vector used as templates. The forward primer, (5′) GCATGCCATGGC CCACTTCC, and the reverse primer described above were used in the PCR. Tax mutant M1 was constructed by PCR-based site-directed mutagenesis with (5′) GACATGCCATGGCCTCCTTCCCAGGGTTTGG as the forward primer and (5′) GTCGCGGATCCTCAGACTTCTGTTTCTCG as the reverse primer and with wild-type Tax cDNA as the template. M1, M7, M22, and M47 were then cloned into the pET-11d vector for expression. Tax(1–244) was generated by inserting an *Xba*I termination linker at the *Stu*I restriction site in the wild-type Tax sequence. Tax(1–319) was generated by linearizing pET-M47 with the *Bgl*II restriction enzyme to cause a termination of transcription and translation at amino acid residue 319.

Gel electrophoretic mobility shift assay. A ^{32}P-labeled *Bgl*II-*Nco*I fragment containing the TATA-proximal 21-bp repeat (47 bp) was prepared by filling in the protruding ends in the presence of [α-^{32}P]dCTP and Klenow enzyme. After electrophoresis in an 8% polyacrylamide gel in Tris-borate-EDTA buffer, the labeled fragment was eluted by soaking the gel slices in double-distilled water and was used for gel shift analysis. The DNA binding reaction mixture typically consisted of approximately 10 pg (4,000 cpm) of radiolabeled DNA fragment, 0.5 to 1 μg of poly(dI-dC), 0.1 μg of purified CREB, and approximately 0.1 μg of purified Tax (42) or GST-Tax in 1× binding buffer (25 mM HEPES [*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; pH 7.9], 5 mM KCl, 0.5 mM EDTA, 1 mg of bovine serum albumin [BSA] per ml, 10% glycerol, and 0.25 mM dithiothreitol). To remove the GST moiety from GST-Tax, the fusion protein was incubated with 0.2 U of thrombin (Sigma) for 30 min at 37°C and the treated protein was then incubated with CREB and DNA in the binding reaction mixture. After incubation for 30 min at 37°C, the binding reaction mixture was chilled on ice and subjected to electrophoresis in a 4% nondenaturing polyacrylamide gel (42). The gel was subsequently dried and autoradiographed.

Protein purification. Bacterial cells (BL21DE3) containing the pET-11a-CREB plasmid were grown in Luria-Bertani broth containing 100 μg of ampicillin per ml, and CREB expression was induced with IPTG. Purification of CREB protein was carried out with DNA cellulose column chromatography (43). GST-CREB, GST-Tax, GST-M1, GST-M7, GST-Tax(96–353), and GST were purified from HB101 cells containing the respective expression constructs after IPTG induction. Glutathione-agarose was then used for protein purification.

Affinity precipitation with a glutathione matrix. Glutathione-Sepharose was prepared in BBN (gel shift binding buffer supplemented with 0.15% Nonidet P-40, 1 mg of BSA per ml, and 0.5 mM phenylmethylsulfonyl fluoride). Approximately 500 ng of purified GST and GST-CREB was incubated with glutathione-Sepharose (60 μl of 50% slurry) for 1 h at 4°C. The GST-charged Sepharose was then washed once with BBN to remove unbound proteins and resuspended in 300 μl of BBN. The TNT T7 coupled reticulocyte lysate system from Promega was used to translate the wild-type and mutant Tax proteins in vitro. Plasmid DNA (1 μg) in a mixture of 50 μl of 50% lysate and 40 μCi of [^{35}S]methionine (>1,000 Ci/mmol [Amersham]) was used to express mutant and wild-type proteins from the bacteriophage T7 promoter. Ten microliters of each of the translation reaction mixtures was added to two sets of glutathione-Sepharose beads charged with GST or GST-CREB. The mixture was then incubated at 4°C for 2 h with shaking. The Sepharose was then washed four times, each with 200 μl of BBN. In vitro-translated proteins that remained bound to the beads via GST or GST-CREB were then eluted in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer and subjected to electrophoresis on an SDS-12% polyacrylamide gel. The gel was then soaked for 30 min in 1 M sodium salicylate, dried, and exposed for autoradiography.

Biotinylated-DNA precipitation. Streptavidin-agarose was prepared in BBN containing 1 mg of BSA per ml to reduce nonspecific adsorption of labeled proteins. The DNA fragment containing two TATA-proximal copies of the 21-bp repeats (2×21) was biotinylated by filling in the protruding ends in the presence of d(C,G,T)TP, biotin-dATP (biotin and the dATP moieties were separated by a 14-carbon spacer), and Klenow enzyme. The biotinylated 2×21 DNA was ethanol precipitated and redissolved in 100 μl of water; 6 μl of the biotinylated DNA fragment was then used in each reaction mixture. Wild-type and mutant Tax proteins were translated in vitro as described above. The DNA binding reaction mixture consisted of 10 μl of translated Tax proteins, 6 μl of biotinylated 2×21 DNA (approximately 10 ng), and 0.1 μg of purified CREB in 1× binding buffer. The binding reaction mixture was incubated for 30 min at 37°C and then added to streptavidin-agarose resuspended in 300 μl of BBN. The reaction mixture was incubated for an additional 2 h at 4°C on a rotator. The beads were then washed with 200 μl of BBN four times. In vitro-labeled Tax proteins retained on the beads via formation of a ternary complex with CREB and the 2×21 DNA were then eluted in SDS sample buffer and analyzed as described above.

Immunoprecipitation. Protein A-Sepharose was preincubated in BBN supplemented with 1 mg of BSA per ml to reduce nonspecific binding of labeled protein. Purified Tax (0.4 μg), GST-Tax (0.4 μg), and GST-Tax after thrombin treatment [GST-Tax(thr.) (0.4 μg)] were incubated with 8 μl of in vitro-translated ^{35}S-labeled CREB and Tax-C-Ab (5 μl) in gel shift binding buffer for 30 min at 37°C. The reaction mixture was then added to 50 μl of 50% protein A–Sepharose slurry suspended in 200 μl of BBN. The reaction mixture was subsequently incubated for 2 h at 4°C with constant shaking. The beads were then washed five times with 200 μl of BBN. The bound protein was eluted in SDS buffer, subjected to electrophoresis on a 12% polyacrylamide gel, soaked in 1 M sodium salicylate, dried, and exposed for autoradiography. Coimmunoprecipitation of in vitro-translated ^{35}S-labeled SRF with various forms of Tax was carried out similarly.

Chloramphenicol acetyltransferase assay. Jurkat cells were transfected with p218-CAT (17) containing two copies of the HTLV-I 21-bp repeat upstream of the chloramphenicol acetyltransferase reporter alone or with CMV–wild-type

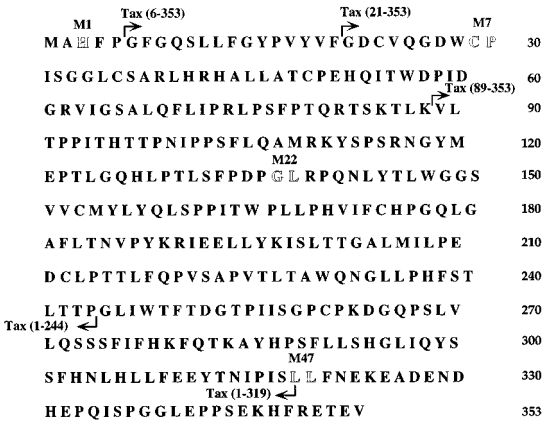

FIG. 1. Amino acid sequences of HTLV-I Tax and its mutants. The starts and stops of the various Tax deletion mutants characterized in this study are indicated by arrows at the relevant positions. The numbers in parentheses above the arrows denote amino acid residues contained within the deletion mutants. M1, M7, M22, and M47 contain H3S, C29A and P30S, G137A and L138S, and L319R and L320S amino acid substitutions, respectively, with the wild-type residues given in open letters. The numerical designations of mutants are given according to the system of Smith and Greene (36).

Tax, CMV-M7, CMV-M22, and CMV-M47 by the DEAE-dextran method as previously described (41). The amount of DNA used in each transfection was held constant at 5.5 μg.

RESULTS

NH_2 terminus of Tax is crucial for interaction with CREB. We have previously reported that Tax interacts directly with the CREB homodimer and CREB–ATF-1 heterodimer via the CREB subunit (43). The multiprotein complexes formed by Tax and CREB or Tax and CREB–ATF-1 assemble with increased efficiency on HTLV-I 21-bp repeats. Notably, the Tax-CREB complex exhibits greatly increased DNA recognition specificity compared with CREB alone. We showed by in vitro binding site selection that Tax-CREB preferentially interacted with a consensus binding site, GGGGG(T/G)TGACG(T/C)(A/C)TA(T/C)CCCCC, which is highly homologous to the HTLV-I 21-bp repeats in general and the promoter-proximal 21-bp repeat in particular (31). In contrast, CREB alone binds only to a consensus motif, GNTGACG(T/C), lacking the G- and C-rich flanking sequences. The G- and C-rich flanking sequences are required for the stable assembly of the Tax-CREB complex in vitro and transactivation by Tax in vivo. That the physical presence of Tax in the ternary complex is crucial for transactivation supports the notion that Tax contains distinct domains which interact with CREB and the basal transcriptional machinery. To localize the regions in Tax responsible for binding CREB, successive deletions in the NH_2- and COOH-terminal coding sequences of Tax were made. The interaction of truncated Tax proteins with CREB was then measured by two assays. The first involved the capture of various in vitro-translated, [^{35}S]methionine-labeled Tax proteins by GST-CREB fusion protein bound to the glutathione-agarose matrix. The second involved the assembly of a ternary complex containing [^{35}S]methionine-labeled Tax–CREB–biotinylated HTLV-I 21-bp repeats and its precipitation by the streptavidin-conjugated Sepharose beads. Figure 1 shows the amino acid sequence of HTLV-I Tax and the positions of the NH_2- and COOH-terminal truncations and the various amino acid substitution mutants that we analyzed. The [^{35}S]methionine-labeled wild-type and truncated Tax proteins were synthesized by in vitro translation (Fig. 2, lanes 1, 4, 7, 10, 13, 16, and 19) and incubated with purified GST or GST-CREB proteins. This step was followed by incubation with glutathione-Sepharose beads. As indicated, GST-CREB but not GST allowed the efficient capture of wild-type Tax on glutathione-Sepharose (lanes 1 to 3). As expected, the luciferase control bound neither GST nor GST-CREB (lanes 20 and 21). Any perturbations in the NH_2-terminal region of Tax (lanes 4 to 12), including a small deletion of six amino acid residues (lanes 4 to 6), obliterated the interaction with CREB. In contrast, deletion of 34 amino acid residues from the COOH terminus of Tax did not interfere with Tax-CREB interaction (lanes 13 to 15). However, when the deletion extended into amino acid residue 245 of Tax, the protein-protein interaction became greatly impaired. Because Tax, CREB, and the HTLV-I 21-bp repeats assemble a ternary complex (43), we also used this as a criterion to characterize the various Tax mutants. A 76-bp *Nco*I-*Xho*I DNA fragment containing two HTLV-I 21-bp repeats was labeled with biotinylated dATP and used in the binding reactions with the Tax-CREB complex. The ternary complex was then precipitated with streptavidin-agarose. As anticipated, in the presence of CREB, ^{35}S-labeled wild-type

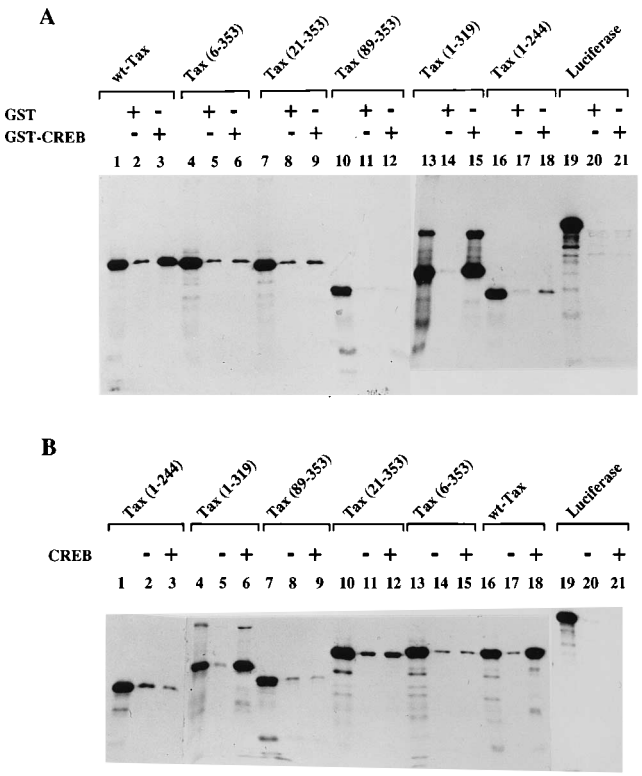

FIG. 2. NH_2 terminus of Tax is crucial for interaction with CREB. In vitro-translated wild-type (wt) and mutant Tax proteins analyzed in each assay are listed at the top of their respective lanes. (A) Affinity precipitation with the glutathione matrix was carried out as described in Materials and Methods. In vitro-translated proteins (lanes 1, 4, 7, 10, 13, 16, and 19) were coprecipitated with GST (lanes 2, 5, 8, 11, 14, 17, and 20) or GST-CREB (lanes 3, 6, 9, 12, 15, 18, and 21). The translated proteins retained on the glutathione-Sepharose matrix via interaction with GST or GST-CREB were dissolved in SDS sample buffer, subjected to SDS-PAGE (12% polyacrylamide), dried, and autoradiographed. (B) Biotinylated DNA precipitation. An 80-bp DNA fragment containing HTLV-I 21-bp repeats was biotinylated. This biotinylated 2×21 DNA and in vitro-translated Tax proteins (lanes 1, 4, 7, 10, 13, 16, and 19) were incubated with (lanes 3, 6, 9, 12, 15, 18, and 21) or without (lanes 2, 5, 8, 11, 14, 17, and 20) CREB at 37°C for 30 min. The reaction mixtures were subsequently incubated with streptavidin-agarose. In vitro-translated Tax proteins that bound to CREB at the DNA were precipitated, electrophoresed, dried, and autoradiographed.

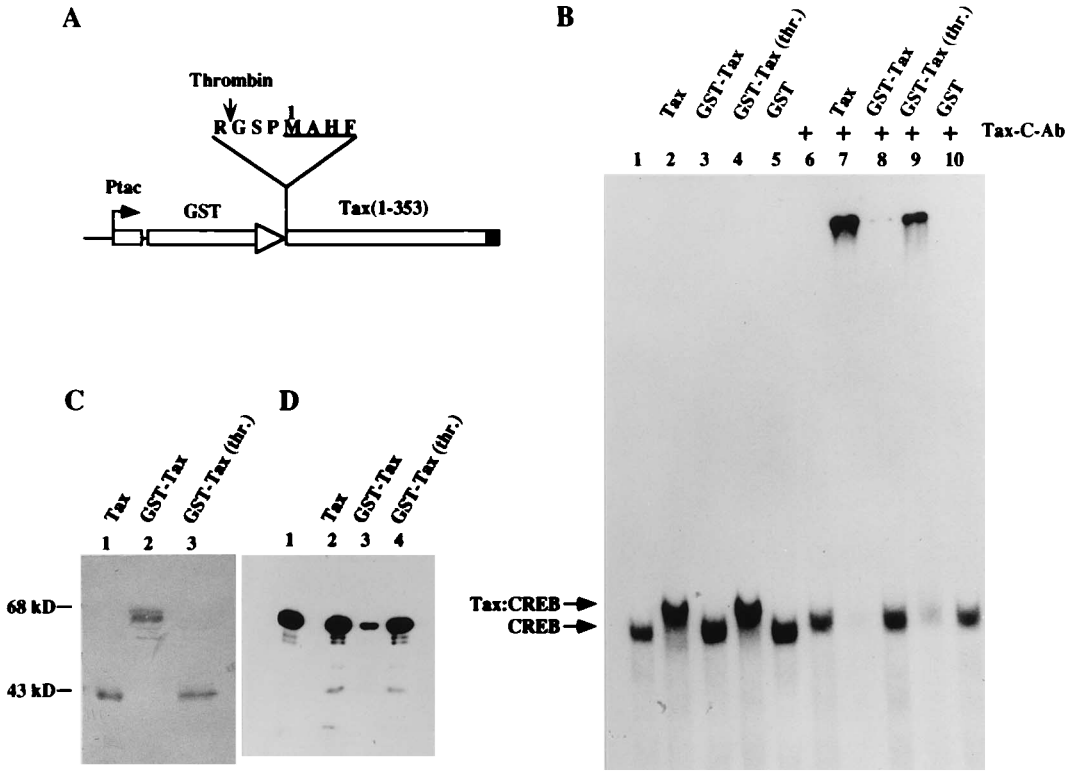

FIG. 3. Attachment of the GST moiety to the NH_2 terminus of Tax blocks interaction with CREB. (A) Construction of a GST-Tax fusion protein. Amino acid residues at the GST-Tax junction of the fusion protein are shown above the diagram. Residues from Tax are underlined, with the first methionine marked. The thrombin cleavage site is marked with an arrow. The solid box at the COOH terminus of Tax indicates the appendage of a six-histidine extension described previously (42). (B) Gel electrophoretic mobility shift assays were carried out as described in Materials and Methods. ^{32}P-labeled 21(1) and purified CREB were incubated without (lanes 1 and 6) or with (lanes 2 and 7) purified Tax, GST-Tax (lanes 3 and 8), GST-Tax(thr.) (lanes 4 and 9), or GST (lanes 5 and 10). GST-Tax(thr.) represents Tax released from the fusion protein after proteolytic cleavage by thrombin. Nucleoprotein complexes containing CREB or Tax-CREB (Tax:CREB) are indicated by arrows. Tax-C-Ab (1 μl [lanes 6 to 10]) was added to confirm the presence of Tax in the ternary complexes. (C) Western blot of purified Tax, GST-Tax, and GST-Tax after thrombin treatment [GST-Tax(thr.); lanes 1 to 3, respectively]. The amounts shown are used for both immunoprecipitation and gel shifts. (D) Immunoprecipitation of in vitro-translated CREB (lane 1) with Tax, GST-Tax, and GST-Tax(thr.) (lanes 2 to 4, respectively).

Tax became bound to the affinity matrix (Fig. 2B, lane 18). In the absence of CREB, little binding of Tax to the streptavidin-agarose was detectable (Fig. 2B, lane 17). The ability of the truncated Tax proteins to participate in ternary complex formation correlated well with that observed with the GST-CREB assay; i.e. NH_2-terminal alterations of Tax resulted in abrogation of ternary complex assembly (Fig. 2B, lanes 7 to 15), while deletion of 34 amino acid residues from the COOH terminus did not impair the ability of Tax to form ternary complexes (Fig. 2B, lanes 4 to 6). Large truncations of COOH-terminal sequence [Tax(1–244), lanes 1 to 3], however, abolished ternary protein-protein-DNA interactions. These results indicate that the NH_2 terminus of Tax is critical for its interaction with CREB. Furthermore, although a relatively short deletion in the COOH terminus of Tax did not affect this interaction, a large truncation [as in Tax(1–244)] abolished the ability of Tax to engage CREB effectively.

Attachment of the GST moiety to the NH_2 terminus of Tax blocks interaction with CREB. As part of an effort to characterize Tax-CREB interaction, a GST-Tax fusion protein which contained the GST moiety fused to the NH_2 terminus of full-length Tax was prepared (Fig. 3A). The GST-Tax fusion protein was purified and analyzed in the gel electrophoretic mobility shift assay together with CREB and the promoter-proximal HTLV-I 21-bp repeat, 21(1). In contrast to the wild-type Tax, GST-Tax failed to form a more slowly migrating complex with CREB and the 21(1) DNA (compare lanes 2 and 3 in Fig. 3B). Taking advantage of the presence of a thrombin cleavage site at the fusion junction in GST-Tax (Fig. 3A), we removed the GST moiety from Tax by proteolysis. As indicated in Fig. 3C, after thrombin treatment, GST-Tax (lane 2) was converted to a form [GST-Tax(thr.)] containing only a short peptide extension in the NH_2 terminus (lane 3) that comigrated with the wild-type Tax (lane 1). Much like the wild-type Tax, this version of Tax was able to assemble a more slowly migrating ternary complex with CREB and the probe DNA (Fig. 3B, lane 4). The presence of Tax in the ternary complex was further demonstrated by the Tax-C-Ab supershift (compare lanes 8 and 9 before and after thrombin cleavage). Finally, GST-Tax failed to coprecipitate with CREB, but thrombin treatment restored the coprecipitation (Fig. 3D, lanes 3 and 4). These results suggest the importance of the NH_2 terminus of Tax in the assembly of the Tax-CREB-21(1) ternary complex. Apparently, the GST domain in the NH_2 terminus of Tax presented a steric hindrance of Tax-CREB interaction which was removed by thrombin proteolysis.

Biochemical characterizations of amino acid substitution mutants of Tax support the presence of distinct functional domains in Tax. Smith and Greene (36) have previously produced a large panel of amino acid substitution mutants of Tax. These mutants could be classified into three categories: (i) mutants that were unable to activate transcription from either

NF-κB binding sites in the HIV LTR or the CRE in the HTLV-I 21-bp repeats [These mutants include M7 (C29A, P30S), which was impaired in localization to the nucleus.]; (ii) mutants M1 (H3S) and M47 (L319R, L320S) that activated transcription from NF-κB sites but not HTLV-I 21-bp repeats; and (iii) a mutant, M22 (G137A, L138S), which activated transcription from the HTLV-I 21-bp repeats with reduced efficiency but was defective in transactivation from the NF-κB binding site. We placed the coding sequences of all four mutants under the control of the bacteriophage T7 promoter. They were then expressed and radiolabeled by in vitro translation as described above and tested for interaction with CREB. Mutant M7 failed to interact with GST-CREB or assemble a ternary complex with CREB and the biotinylated 21-bp repeats (Fig. 4A and B, lanes 5 and 6). Although mutant M1 interacted somewhat with GST-CREB (Fig. 4A, lanes 14 and 15), it was unable to assemble a ternary complex with CREB and the biotinylated 21-bp-repeat DNA (Fig. 4B, lanes 14 and 15) and thus failed to transactivate the HTLV-I LTR. The defects in these two mutants are not due to gross conformational alterations from the amino acid substitutions because they both interact with SRF p67 like the wild-type Tax (Fig. 4C, lanes 3 and 5). Consistent with its ability to interact with SRF in vitro, M1 was observed to activate expression from the c-*fos* promoter, M7, however, was unable to activate the c-*fos* promoter, most likely because of a nuclear localization defect (not shown).

In contrast and as anticipated, M22 interacted with CREB positively but with reduced affinity (Fig. 4A and B, lanes 8 and 9). Most notably, M47 interacted with CREB better than M22 (Fig. 4A, lanes 11 and 12) and assembled stably into the Tax-CREB-21(1) ternary complex (Fig. 4B, lanes 11 and 12). Apparently, the deficiency of M47 in transactivating the HTLV-I LTR in vivo was not due to a defect in Tax-CREB interaction but most likely resulted from an impairment in steps critical for transcriptional activation that occurred after the assembly of Tax-CREB complex on the HTLV-I enhancer/promoter. These results are consistent with the previous reports from Fujii et al. (12) and Semmes and Jeang (35) showing the presence of a transactivation domain in Tax which, when fused to the Gal4 DNA binding domain, was able to activate transcription from the Gal4 binding site. The results from both the GST-CREB assay and the ternary complex formation assay together with the in vivo phenotypes of the amino acid substitution mutants are listed in Fig. 5 for comparison. Because M47 presumably can tie up CREB in forming an inactive complex, we tested if M47 exhibited a *trans*-dominant negative phenotype over the wild-type Tax by cotransfection. Surprisingly, increasing amounts of M47 had no effect on the transactivation of the 21-bp repeats by wild-type Tax (lanes 6 to 9). This is not due to a proteolytic degradation of M47, because the M47 expression levels are comparable to those of the wild-type Tax (36). Increasing amounts of M7, however, exerted a negative effect (lanes 10 to 13) on Tax transactivation. We interpret these results to suggest that the intracellular level of CREB protein is not limiting. Hence, the formation of a nonfunctional Tax (M47)-CREB complex did not significantly affect the level of the functional Tax (wild-type)-CREB complex and transactivation. On the other hand, the basal transcriptional components required for transactivation are probably limiting. As a result, even though M7 is defective in nuclear localization, it probably interacted with and rendered unavailable the basal factors for interaction with the wild-type Tax.

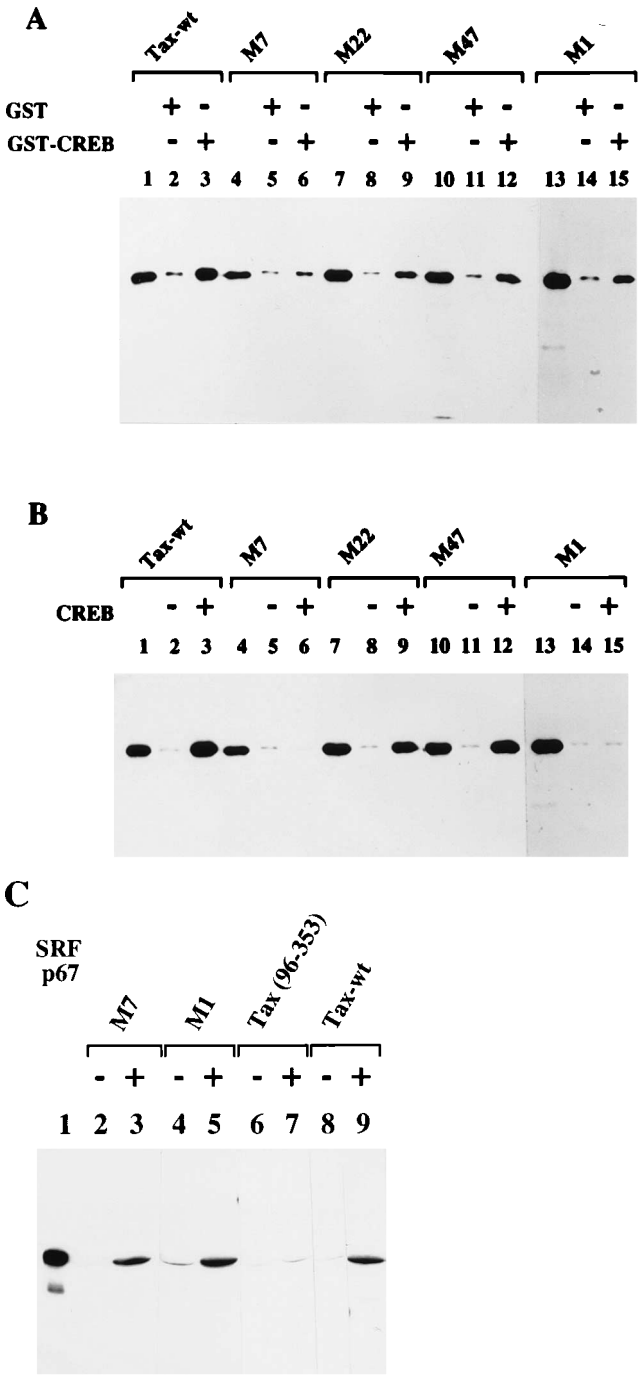

FIG. 4. Interaction of mutants M1, M7, M22, and M47 with CREB and HTLV-I 21-bp repeats. In vitro-translated wild-type (wt) and mutant Tax proteins analyzed are marked at the top of their respective lanes. (A) Affinity precipitation with the glutathione matrix as described in the legend to Fig. 2. In vitro-translated proteins (lanes 1, 4, 7, 10, and 13) were precipitated either with GST (lanes 2, 5, 8, 11, and 14) or with GST-CREB (lanes 3, 6, 9, 12, and 15). (B) Precipitation with biotinylated 2×21 DNA as described in the legend to Fig. 2. In vitro-translated proteins (lanes 1, 4, 7, 10, and 13) were precipitated in the absence (lanes 2, 5, 8, 11, and 14) or presence (lane 3, 6, 9, 12, and 15) of purified CREB. (C) Coimmunoprecipitation of in vitro-translated ^{35}S-labeled SRF p67 (lane 1) was performed with Tax-C-Ab in the presence or absence of wild-type Tax (lanes 8 and 9), M1 (lanes 4 and 5), M7 (lanes 2 and 3), and Tax(96–353) (lanes 6 and 7). Wild-type Tax and Tax mutants were generated by thrombin cleavage of their respective GST fusion proteins (as described in the legend to Fig. 3).

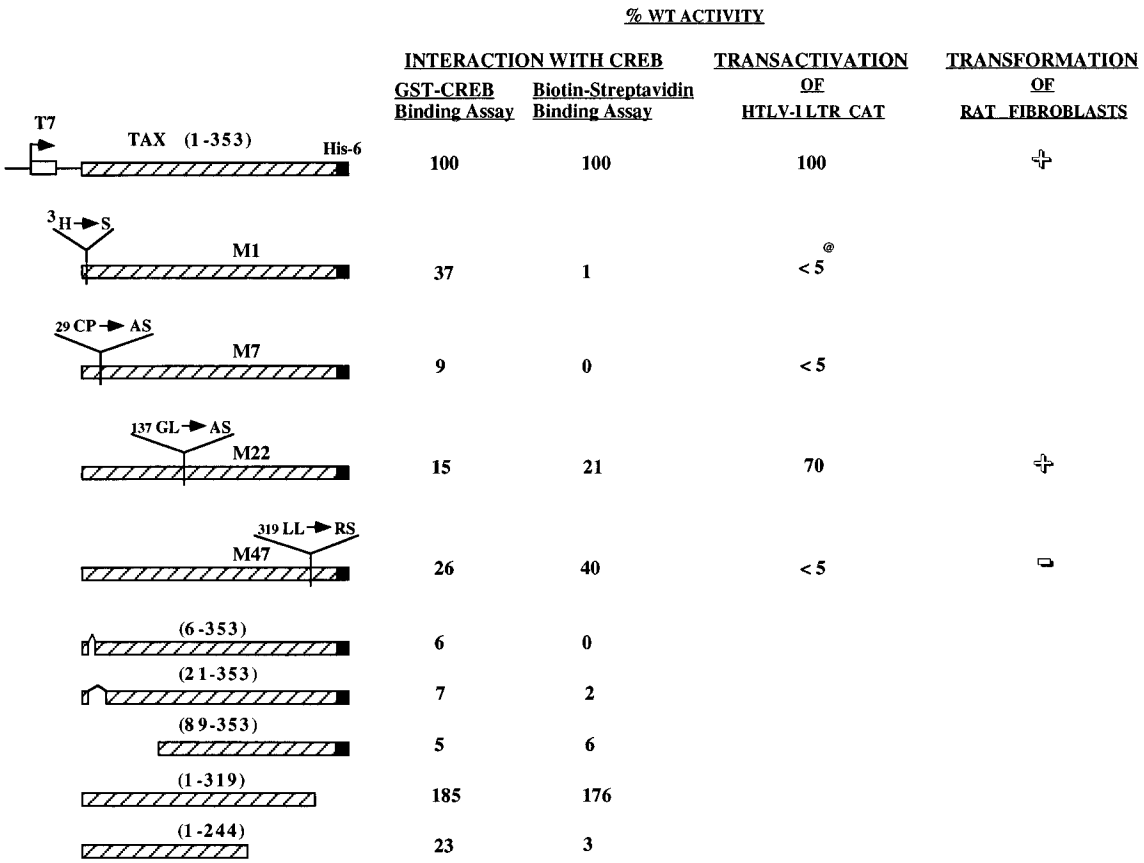

FIG. 5. In vitro characterizations of Tax mutants and correlation with transactivation of HTLV-I LTR and cellular transformation. Wild-type Tax and various deletion and amino acid substitution mutants were expressed from the bacteriophage T7 promoter. The Tax mutants examined are as described in the legend to Fig. 1. The glutathione-Sepharose assay and streptavidin-agarose assay were used to characterize the Tax mutants (Fig. 2 and 4). The percentages of wild-type (wt) activities of each mutant are averages from three independent experiments normalized against the levels of input labeled proteins. Transactivation of the HTLV-I LTR chloramphenicol acetyltransferase (CAT) construct was carried out by transfection of Jurkat cells by the DEAE-dextran method. These results confirmed previous findings in a report by Smith and Greene (36). Data about the transformation of rat fibroblasts (Rat-2 cells) by various Tax proteins were derived from another study by Smith and Greene (37). @, datum derived from a study by Smith and Greene (36).

DISCUSSION

We reported earlier that transcriptional activation via the HTLV-I 21-bp repeats by Tax is mediated by a direct protein-protein interaction between Tax and the homodimeric and heterodimeric (CREB–ATF-1) forms of the ubiquitous transcription factor CREB (43). The Tax-CREB complex selectively interacts with a specific subset of CRE motifs containing long runs of G-rich and/or C-rich sequences in the 5′- and 3′-flanking regions present in the viral 21-bp repeats (31). The preferential assembly of the Tax-CREB complex on the HTLV-I 21-bp repeats and the resulting physical presence of Tax on the HTLV-I enhancer are crucial for Tax transactivation. Apparently, these specific protein-nucleic acid interactions allow the transactivation domain of Tax to be tethered indirectly to the HTLV-I enhancer for transcriptional augmentation. Most recently, we found that Tax specifically engaged three amino acid residues, ^{282}AAR^{284}, near the conserved DNA binding domain (^{289}RXXKNRXXAAXXCRXRK^{305}) of CREB in forming the Tax-CREB complex (1). These data further strengthen the notion that the interaction between Tax and CREB results in a complex with an altered DNA recognition property. In this report, we show that the integrity of the NH_2-terminal domain of Tax is critical for this interaction. Most if not all alterations in the NH_2 terminus of Tax abrogated Tax-CREB interaction in vitro. The importance of this region for CREB interaction is further emphasized by a previous report showing its involvement in determining the specificity of HTLV-I Tax and HTLV-II Tax for activating the respective viral LTRs (5). The analyses of amino acid substitution mutants of Tax described here coincide with in vivo characterizations published previously (36) and further reveal that the formation of the Tax–CREB–21-bp-repeat ternary complex is a necessary first step toward Tax transactivation. Consistent with this notion, mutant M1 interacted with CREB to some degree but failed to assemble a stable ternary complex with CREB and the 21-bp repeats and thus was unable to bring about transactivation. Our results support the idea that the recruitment of Tax via CREB to the HTLV-I enhancer facilitates the interaction between the transactivation domain of Tax and the basal transcriptional machinery, which in turn leads to increased HTLV-I gene expression. This is best indicated by the mutant Tax M47 (L319R, L320S), which interacted with CREB and HTLV-I 21-bp repeats in vitro but nevertheless was unable to activate the HTLV-I enhancer in vivo. The defect in M47 most likely resulted from a lesion in the transactivation domain. Indeed, the presence of such a domain in Tax has been suggested (12). In excellent agreement with the data described here, but with a different approach, Semmes and Jeang (35a) demonstrate that the amino acid residues 284 to 322 in the COOH-terminal region of Tax con-

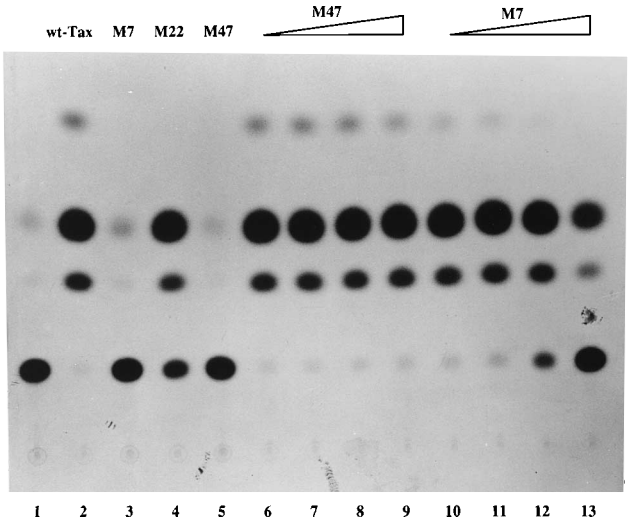

FIG. 6. Transactivation of HTLV-I 2×21 CAT (chloramphenicol acetyltransferase) DNA by the Tax mutants M7, M22, and M47 and competition of the wild type with M47 and M7. The reporter plasmid (1 μg) was transfected into Jurkat cells either alone or in conjunction with wild-type (wt) Tax, M7, M22, and M47 (lanes 1 to 5, respectively). All constructs are expressed from the CMV promoter. Lanes 6 to 9 show cotransfections of wild-type Tax and M47. The wild-type Tax DNA was held constant at 0.5 μg; the amounts of M47 were 0.5, 1, 2, and 4 μg (lanes 6 to 9, respectively). Lanes 10 to 13 show the results of cotransfection with wild-type Tax and M7. The amount of Tax DNA was 0.5 μg, while the amounts of M7 DNA were 0.5, 1, 2, and 4 μg (lanes 10 to 13, respectively).

stitute a transactivation domain. M47 did not compete with the wild-type Tax in a *trans*-dominant negative manner, possibly because M47 interaction with CREB is weakened and/or the intracellular availability of CREB is not limiting. In contrast, M7, a mutant Tax that did not interact with CREB in vitro and was partially impaired in nuclear localization, competed somewhat with the wild-type Tax (Fig. 6). We interpret this result to suggest that M7 titrated out limiting basal transcription factors via its functional transactivation domain, a notion consistent with results reported by Semmes and Jeang (35a). It is interesting that a direct interaction between Tax and TATA binding protein has been reported recently (6). The amino acid substitutions in M47, however, did not affect its interaction with TATA binding protein, suggesting that additional basal transcriptional factors may be involved in bringing about the eventual transactivation. Our results indicate that, much like other virus transactivators such as E1a and VP16, Tax contains multiple domains involved in interacting with various components of the eukaryotic machinery. Finally, despite the presence of an acidic domain in the COOH-terminal end of Tax, this region could be deleted without affecting Tax function as previously demonstrated (36).

Transgenic mice harboring the *tax* transgene have been found to develop neurofibromas and multiple mesenchymal tumors (14, 19, 28). Furthermore, Tax is able to transform NIH 3T3 cells (40) and Rat-2 cells (37) in culture. Like c-*myc* and adenoviral E1a, Tax collaborates with activated Ras to transform rat embryo fibroblast cells (33). Interestingly, Smith and Greene found that the oncogenicity of Tax in rat fibroblasts was linked to its ability to transactivate through the CREB-ATF but not the NF-κB pathway; i.e., Tax mutant M22 but not M47 transformed. The data presented here suggest that the transcriptional induction of specific cellular genes via a productive interaction between Tax and CREB may be ultimately responsible for cellular transformation.

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REFERENCES

1. **Adya, N., L. J. Zhao, H. Wan, I. Boros, and C. Z. Giam.** 1994. Expansion of CREB's DNA recognition specificity by HTLV-I Tax results from interaction with ^{282}Ala Ala Arg near the conserved DNA binding domain of CREB. Proc. Natl. Acad. Sci. USA **91:**5642–5646.
2. **Béraud, C., S.-C. Sun, P. Ganchi, D. W. Ballard, and W. C. Greene.** 1994. Human T-cell leukemia virus type I Tax associates with and is negatively regulated by the NF-κB2 p100 gene product: implications for viral latency. Mol. Cell. Biol. **14:**1374–1382.
3. **Bhagavati, S., G. Ehrlich, R. W. Kula, S. Kwok, J. Sninsky, V. Udani, and B. J. Poiesz.** 1988. Detection of human T-cell lymphoma/leukemia virus type I DNA and antigen in spinal fluid and blood of patients with chronic progressive myelopathy. N. Engl. J. Med. **318:**1141–1147.
4. **Brady, J., K.-T. Jeang, J. Duvall, and G. Khoury.** 1987. Identification of p40x-responsive regulatory sequences within the human T-cell leukemia virus type I long terminal repeat. J. Virol. **61:**2175–2181.
5. **Cann, A. J., J. D. Rosenblatt, W. Wachsman, and I. S. Y. Chen.** 1989. In vitro mutagenesis of the human T-cell leukemia virus types I and II *tax* genes. J. Virol. **63:**1474–1479.
6. **Caron, C., R. Rousset, C. Beraud, V. Moncollin, J. M. Egly, and P. Jalinot.** 1993. Functional and biochemical interaction of the HTLV-I Tax 1 transactivator with TBP. EMBO J. **12:**4269–4278.
7. **Chen, I. S., D. J. Slamon, J. D. Rosenblatt, N. P. Shah, S. G. Quan, and W. Wachsman.** 1985. The x gene is essential for HTLV replication. Science **229:**54–58.
8. **Crenon, I., C. Beraud, P. Simard, J. Montagne, P. Veschambre, and P. Jalinot.** 1993. The transcriptionally active factors mediating the effect of the HTLV-I Tax transactivator on the IL-2R alpha kappa B enhancer include the product of the c-rel proto-oncogene. Oncogene **8:**867–875.
9. **Felber, B. K., H. Paskalis, C. Kleinman Ewing, F. Wong Staal, and G. N. Pavlakis.** 1985. The pX protein of HTLV-I is a transcriptional activator of its long terminal repeats. Science **229:**675–679.
10. **Fujii, M., P. Sassone Corsi, and I. M. Verma.** 1988. c-fos promoter transactivation by the tax1 protein of human T-cell leukemia virus type I. Proc. Natl. Acad. Sci. USA **85:**8526–8530.
11. **Fujii, M., H. Tsuchiya, T. Chuhjo, T. Akizawa, and M. Seiki.** 1992. Interaction of HTLV-1 Tax1 with p67SRF causes the aberrant induction of cellular immediate early genes through CArG boxes. Genes Dev. **6:**2066–2076.
12. **Fujii, M., H. Tsuchiya, and M. Seiki.** 1991. HTLV-1 Tax has distinct but overlapping domains for transcriptional activation and for enhancer specificity. Oncogene **6:**2349–2352.
13. **Fujisawa, J.-I., M. Toita, and M. Yoshida.** 1989. A unique enhancer element for the *trans* activator (p40^{tax}) of human T-cell leukemia virus type I that is distinct from cyclic AMP- and 12-*O*-tetradecanoylphorbol-13-acetate-responsive elements. J. Virol. **63:**3234–3239.
14. **Furuta, Y., S. Aizawa, Y. Suda, Y. Ikawa, H. Kishimoto, Y. Asano, T. Tada, A. Hikikoshi, M. Yoshida, and M. Seiki.** 1989. Thymic atrophy characteristic in transgenic mice that harbor pX genes of human T-cell leukemia virus type I. J. Virol. **63:**3185–3189.
15. **Gessain, A., F. Barin, J. C. Vernant, O. Gout, L. Maurs, A. Calender, and G. DeThe.** 1985. Antibodies to human T-lymphotropic virus type-I in patients with tropical spastic paraparesis. Lancet **2:**407–410.
16. **Giam, C. Z., M. Nerenberg, G. Khoury, and G. Jay.** 1986. Expression of the complete human T-cell leukemia virus type I pX coding sequence as a functional protein in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **83:**7192–7196.
17. **Giam, C. Z., and Y. L. Xu.** 1989. HTLV-I tax gene product activates transcription via preexisting cellular factors and cAMP responsive element. J. Biol. Chem. **264:**15236–15241.
18. **Gitlin, S. D., P. F. Lindholm, S. J. Marriott, and J. N. Brady.** 1991. Transdominant human T-cell lymphotropic virus type I TAX_1 mutant that fails to localize to the nucleus. J. Virol. **65:**2612–2621.
19. **Hinrichs, S. H., M. Nerenberg, R. K. Reynolds, G. Khoury, and G. Jay.** 1987. A transgenic mouse model for human neurofibromatosis. Science **237:**1340–1343.
20. **Hinuma, Y., H. Komoda, T. Chosa, T. Kondo, M. Kohakura, T. Takenaka, M. Kikuchi, M. Ichimaru, K. Yunoki, I. Sato, R. Matsuo, Y. Takiuchi, H. Uchino, and M. Hanaoka.** 1982. Antibodies to adult T-cell leukemia-virus-associated antigen (ATLA) in sera from patients with ATL and controls in Japan: a nation-wide sero-epidemiologic study. Int. J. Cancer **29:**631–635.
21. **Hirai, H., J. Fujisawa, T. Suzuki, K. Ueda, M. Muramatsu, A. Tsuboi, N. Arai, and M. Yoshida.** 1992. Transcriptional activator Tax of HTLV-1 binds to the NF-kappa B precursor p105. Oncogene **7:**1737–1742.
22. **Inoue, J., M. Seiki, T. Taniguchi, S. Tsuru, and M. Yoshida.** 1986. Induction

of interleukin 2 receptor gene expression by p40x encoded by human T-cell leukemia virus type 1. EMBO J. **5:**2883–2888.
23. **Inoue, J., M. Yoshida, and M. Seiki.** 1987. Transcriptional (p40x) and post-transcriptional (p27x-III) regulators are required for the expression and replication of human T-cell leukemia virus type I genes. Proc. Natl. Acad. Sci. USA **84:**3653–3657.
24. **Jacobson, S., C. S. Raine, E. S. Mingioli, and D. E. McFarlin.** 1988. Isolation of an HTLV-1-like retrovirus from patients with tropical spastic paraparesis. Nature (London) **331:**540–543.
25. **Lanoix, J., J. Lacoste, N. Pepin, N. Rice, and J. Hiscott.** 1994. Overproduction of NFKB2 (lyt-10) and c-Rel: a mechanism for HTLV-I Tax-mediated *trans*-activation via the NF-κB signalling pathway. Oncogene **9:**841–852.
26. **Miyatake, S., M. Seiki, M. Yoshida, and K.-I. Arai.** 1988. T-cell activation signals and human T-cell leukemia virus type I-encoded $p40^x$ protein activate the mouse granulocyte-macrophage colony-stimulating factor gene through a common DNA element. Mol. Cell. Biol. **8:**5581–5587.
27. **Montagne, J., C. Beraud, I. Crenon, G. Lombard Platet, L. Gazzolo, A. Sargeant, and P. Jalinot.** 1990. Tax1 induction of the HTLV-I 21 bp enhancer requires cooperation between two cellular DNA-binding proteins. EMBO J. **9:**957–964.
28. **Nerenberg, M., S. H. Hinrichs, R. K. Reynolds, G. Khoury, and G. Jay.** 1987. The tat gene of human T-lymphotropic virus type 1 induces mesenchymal tumors in transgenic mice. Science **237:**1324–1329.
29. **Nyborg, J. K., W. S. Dynan, I. S. Chen, and W. Wachsman.** 1988. Binding of host-cell factors to DNA sequences in the long terminal repeat of human T-cell leukemia virus type I: implications for viral gene expression. Proc. Natl. Acad. Sci. USA **85:**1457–1461.
30. **Osame, M., M. Matsumoto, K. Usuku, S. Izumo, N. Ijichi, H. Amitani, M. Tara, and A. Igata.** 1987. Chronic progressive myelopathy associated with elevated antibodies to human T-lymphotropic virus type I and adult T-cell leukemia like cells. Ann. Neurol. **21:**117–122.
31. **Paca-Uccaralertkun, S., L.-J. Zhao, N. Adya, J. V. Cross, B. R. Cullen, I. M. Boros, and C.-Z. Giam.** 1994. In vitro selection of DNA elements highly responsive to human T-lymphotropic virus type I transcriptional activator, Tax. Mol. Cell. Biol. **14:**456–462.
32. **Poiesz, B. J., F. W. Ruscetti, A. F. Gazdar, P. A. Bunn, J. D. Minna, and R. C. Gallo.** 1980. Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. Proc. Natl. Acad. Sci. USA **77:**7415–7419.
33. **Pozzatti, R., J. Vogel, and G. Jay.** 1990. The human T-lymphotropic virus type I *tax* gene can cooperate with the *ras* oncogene to induce neoplastic transformation of cells. Mol. Cell. Biol. **10:**413–417.
34. **Rosen, C. A., J. G. Sodroski, and W. A. Haseltine.** 1985. Location of cis-acting regulatory sequences in the human T-cell leukemia virus type I long terminal repeat. Proc. Natl. Acad. Sci. USA **82:**6502–6506.
35. **Semmes, O. J., and K.-T. Jeang.** 1992. Mutational analysis of human T-cell leukemia virus type I Tax: regions necessary for function determined with 47 mutant proteins. J. Virol. **66:**7183–7192.
35a. **Semmes, O. J., and K.-T. Jeang.** 1995. Definition of a minimal activation domain in human T-cell leukemia virus type I Tax. J. Virol. **69:**1827–1833.
36. **Smith, M. R., and W. C. Greene.** 1990. Identification of HTLV-I tax trans-activator mutants exhibiting novel transcriptional phenotypes. Genes Dev. **4:**1875–1885.
37. **Smith, M. R., and W. C. Greene.** 1991. Type I human T cell leukemia virus tax protein transforms rat fibroblasts through the cyclic adenosine monophosphate response element binding protein/activating transcription factor pathway. J. Clin. Invest. **88:**1038–1042.
38. **Smith, M. R., and W. C. Greene.** 1992. Characterization of a novel nuclear localization signal in the HTLV-I tax transactivator protein. Virology **187:** 316–320.
39. **Sodroski, J. G., C. A. Rosen, and W. A. Haseltine.** 1984. Trans-acting transcriptional activation of the long terminal repeat of human T lymphotropic viruses in infected cells. Science **225:**381–385.
40. **Tanaka, A., C. Takahashi, S. Yamaoka, T. Nosaka, M. Maki, and M. Hatanaka.** 1990. Oncogenic transformation by the Tax gene of human T-cell leukemia virus type I in vitro. Proc. Natl. Acad. Sci. USA **87:**1071–1075.
41. **Xu, Y. L., N. Adya, E. Siores, Q. S. Gao, and C. Z. Giam.** 1990. Cellular factors involved in transcription and Tax-mediated trans-activation directed by the TGACGT motifs in human T-cell leukemia virus type I promoter. J. Biol. Chem. **265:**20285–20292.
42. **Zhao, L. J., and C. Z. Giam.** 1991. Interaction of the human T-cell lymphotrophic virus type I (HTLV-I) transcriptional activator Tax with cellular factors that bind specifically to the 21-base-pair repeats in the HTLV-I enhancer. Proc. Natl. Acad. Sci. USA **88:**11445–11449.
43. **Zhao, L. J., and C. Z. Giam.** 1992. Human T-cell lymphotropic virus type I (HTLV-I) transcriptional activator, Tax, enhances CREB binding to HTLV-I 21-base-pair repeats by protein-protein interaction. Proc. Natl. Acad. Sci. USA **89:**7070–7074.